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Original Article

Poincaré Plot Width, Morning Urine Norepinephrine Levels, and Autonomic Imbalance in Children With Obstructive Sleep Apnea

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ABSTRACT

BACKGROUND: Obstructive sleep apnea (OSA) in childhood is accompanied by sympathetic overflow unopposed by the parasympathetic tone. Complex methods like power spectral analysis of heart rate variability have been applied to study this imbalance. In this report, width of Poincaré scattergram of the R-R interval (parasympathetic tone) and morning urine norepinephrine concentration (sympathetic activity) were used to assess autonomic imbalance. **METHODS:** Poincaré plot was obtained from the electrocardiographic channel of nocturnal polysomnography and its width was measured, and norepinephrine-to-creatinine concentration ratio was calculated in morning urine specimen. **RESULTS:** Twenty children with obstructive sleep apnea and moderate-to-severe nocturnal hypoxemia (oxygen saturation of hemoglobin [SpO_2] nadir $<90\%$), 24 subjects with mild hypoxemia (SpO_2 nadir $\geq 90\%$), and 11 control subjects were recruited. Children with obstructive sleep apnea and moderate-to-severe hypoxemia had significantly narrower Poincaré plot width (318.7 $\pm$ 139.3 ms) and higher ln-transformed urine norepinephrine-to-creatinine ratio (4.5 $\pm$ 0.6) than control subjects (484.2 $\pm$ 104.4 ms and 3.8 $\pm$ 0.4, respectively; $P < 0.05$). Ln-transformed urine norepinephrine levels were inversely related to Poincaré plot width ($P = 0.02$). **CONCLUSIONS:** Subjects with obstructive sleep apnea and moderate-to-severe nocturnal hypoxemia have enhanced sympathetic activity and reduced parasympathetic drive. Poincaré plot width and urine norepinephrine levels are simple measures of autonomic imbalance in pediatric obstructive sleep apnea.

Key words: Poincaré plot, sleep apnea, sleep-disordered breathing, sympathetic tone, urine norepinephrine

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Introduction

Although the long-term consequences of obstructive sleep apnea (OSA) on cardiovascular health in childhood remain unknown, there is evidence for progressively increasing blood pressure with worsening OSA severity and autonomic imbalance due to sympathetic overflow unopposed by the parasympathetic tone.[1-3] Moreover, persistently increased diurnal sympathetic activity has been demonstrated in children with OSA by peripheral arterial tonometry or autonomic cardiovascular tests.[4,5] Increased nocturnal sympathetic activity has been identified in pediatric patients with sleep apnea using power spectral analysis of heart rate variability during sleep or measurement of morning urine norepinephrine levels.[2,6,7]

Surrogate measures of autonomic imbalance can be useful when treatment interventions for OSA, such as adenotonsillectomy, and their potential beneficial effects are evaluated. However, the methods reported so far in the literature are complex and difficult to interpret (e.g., power spectral analysis of R-R interval variability). In this

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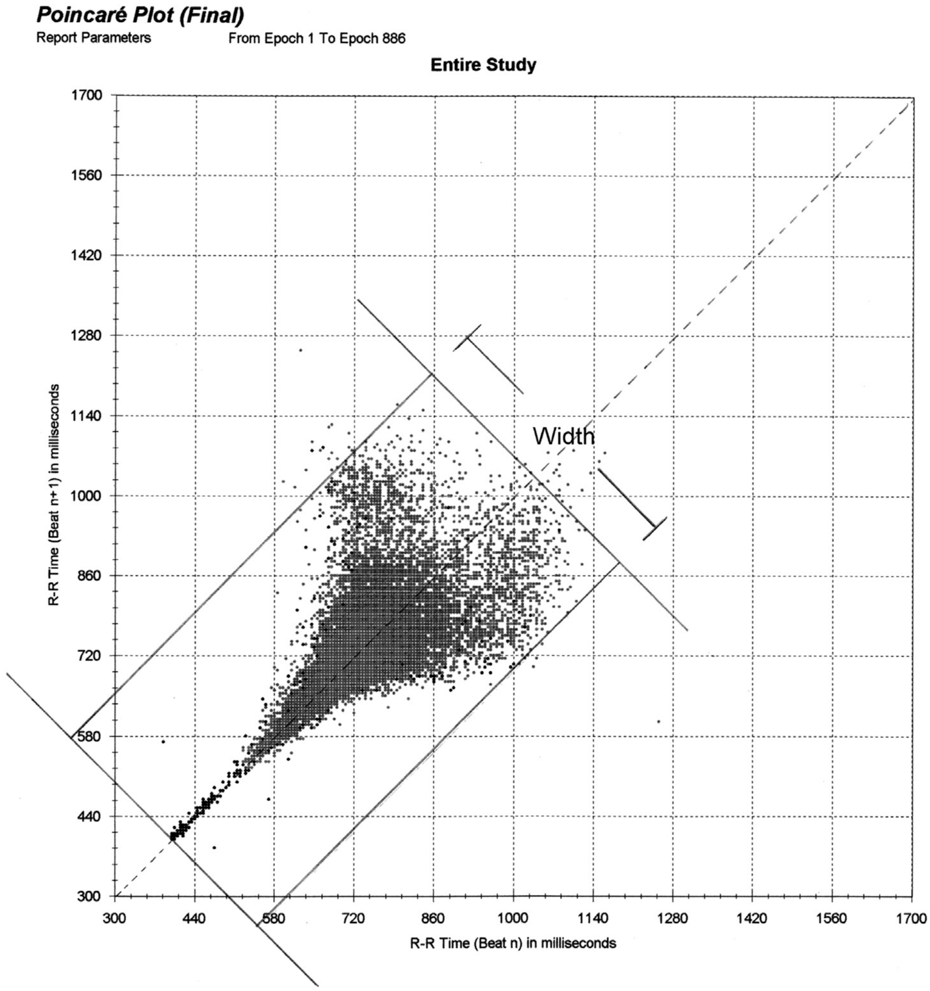

FIGURE 1.
The Poincaré plot of sequential R-R intervals derived from the electrocardiographic channel of polysomnography during the whole night of sleep was assessed after excluding periods of wakefulness. Two lines parallel to the line of identity (diagonal at 45 degrees to both axes), defining the bulk of the plot, were drawn. The distance between the two parallel lines was defined as the width of the plot and was measured graphically. Representative Poincaré scattergram from a patient with obstructive sleep apnea and mild nocturnal hypoxemia (SpO_2 nadir ≥90%) is depicted.

report, width of the Poincaré plot—a scattergram of the R-R interval variability—and morning urine norepinephrine concentration are evaluated as simple indices of autonomic imbalance that might be used as outcome measures in future studies of therapeutic interventions for OSA. Poincaré plot width has been applied in the literature for the quantitative display of parasympathetic nervous system activity.[8] For example, when a subject is in the supine position, parasympathetic activity predominates and the width (scatter) of the Poincaré plot increases, whereas in the standing position parasympathetic drive decreases and the plot becomes narrower.[9]

In the present study, it was hypothesized that (1) children with OSA and moderate-to-severe nocturnal hypoxemia have the narrowest Poincaré plot width and the highest morning urine norepinephrine concentration among subjects with OSA and nocturnal hypoxemia or control participants and (2) if width of the Poincaré scattergram during nocturnal sleep reflects parasympathetic drive and morning urine norepinephrine indicates sympathetic activity, the two variables should be associated inversely with each other.

Patients and Methods

Consecutive children with snoring who were referred for polysomnography and had an apnea-hypopnea index (AHI) of >1 episode/hr were included. Healthy subjects without snoring who underwent polysomnography and had AHI of ≤1 episode/hr were also recruited for the purposes of this study as control subjects. Subjects with cardiovascular, neuromuscular, or genetic disorders or use of antihypertensive medications or bronchodilators were excluded. The study was approved by the Institutional Review Board (Scientific Council; 9258/23-06-10); informed consent was obtained from caregivers and assent from children aged >6 years.

Overnight polysomnography was completed at the Sleep Disorders Laboratory (Somnostar Cephalo Pro; Viasys Healthcare, Yorba Linda, CA). Four-channel electroencephalogram (C3/M2, O2/M1, O1/M2, F4/M1), two-channel electro-oculogram, submental and tibial electromyogram, and electrocardiogram were recorded. The electrocardiographic signal was sampled at 500 Hz. Airflow was detected by thermocouples at the nose and mouth and by nasal pressure transducer, and respiratory movements were monitored using inductive

plethysmography thoracic and abdominal belts (RespiTrace QDC, RIP module; Viasys Healthcare). Oxygen saturation of hemoglobin (SpO_2) was measured by pulse oximetry. Sleep stages, arousals, and respiratory events were scored using the recent American Academy of Sleep Medicine Manual.[10] Respiratory arousals were defined as those occurring within 3 seconds after an apnea, hypopnea, or snore.[11] AHI was the mean number of obstructive, central, and mixed apneas and hypopneas per hour of total sleep time.[10]

The Poincaré plot of sequential R-R intervals derived from the electrocardiographic channel of polysomnography during the whole night of sleep was assessed after excluding periods of wakefulness. To measure the width of the scattergram, two lines parallel to the line of identity (diagonal at 45 degrees to both axes) defining the bulk of the plot were drawn (Fig 1). The distance between the two parallel lines was defined as the width of the Poincaré plot and was measured graphically.

Participants voided before sleep, did not urinate overnight, and provided a urine specimen in the morning. Urine samples were transferred to test tubes and 2-6 drops of HCl solution (6N) were added. Specimens were frozen to −70°C until assayed. Enzyme-linked immunosorbent assay was used to determine urine concentration of norepinephrine (Immuno-Biological Laboratories, Hamburg, Germany) with a lowest detection limit of 0.6 ng/mL. Urine creatinine concentration was measured using routine method.

Width of the Poincaré plot and morning urine norepinephrine concentration were the outcome measures. Urine concentration of norepinephrine was divided by urine concentration of creatinine to adjust for the renal concentrating effect (nanogram of norepinephrine/milligram of creatinine). Sympathetic activity increases by arousal stimuli and also by hypoxemia via stimulation of peripheral and central chemoreceptors especially in the absence of normal airflow.[12,13] For this reason, three groups of participants were formed according to OSA presence and severity of nocturnal hypoxemia: (1) children with OSA and SpO_2 nadir <90% (moderate-to-severe nocturnal hypoxemia); (2) subjects with OSA and SpO_2 nadir ≥90% (mild hypoxemia); and (3) control subjects.[6]

Study groups were compared in terms of demographic characteristics, polysomnography indices, Poincaré plot width, and urine norepinephrine concentration using χ^2 test (categorical data), one-way analysis of variance, followed by post hoc tests for pair comparisons (normally distributed continuous data) or the Kruskal-Wallis test with post hoc Mann-Whitney tests and Bonferroni correction (not normally distributed continuous data). Urine norepinephrine concentration values were ln-transformed to approach a normal distribution.

Pearson correlation was applied to evaluate associations of the Poincaré plot width or ln-transformed urine norepinephrine-to-creatinine ratio with SpO_2 nadir, respiratory arousal index, and AHI. To adjust these associations for age, multiple linear regression analyses were performed including age as independent variable. To assess the relationship between Poincaré plot width and ln-transformed urine norepinephrine-to-creatinine ratio, linear and nonlinear regression models were applied. The model maximizing the squared value of the correlation coefficient and minimizing the standard error of the estimate was selected.

TABLE 1.
Summary Statistics and Significance of Comparisons in Children With Obstructive Sleep Apnea and Control Participants Regarding Subjects' Characteristics, Polysomnography Indices, Poincaré Plot Width, and Morning Urine Norepinephrine Concentration

	OSA With Moderate-to-Severe Hypoxemia (n = 20)	OSA With Mild Hypoxemia (n = 24)	Control Subjects (n = 11)
Age, yr	6.5 ± 3.9	7 ± 2.8	7.5 ± 3
Sex, female (n [%])	6 (30)	7 (29.2)	5 (45.5)
BMI *z* score	1.4 ± 1.3	0.7 ± 1.4	1 ± 1.2
Apnea-hypopnea index, episodes/hr*	15.7 ± 17.4	4.9 ± 4.1	0.5 ± 0.4
Resp. arousal index, episodes/hr*	3 ± 2.8	1.1 ± 1.2	0.3 ± 0.3
Oxygen desaturation (≥3%) of hemoglobin index, episodes/hr*	16.2 ± 19.2	3.7 ± 3.8	1.6 ± 3.3
Oxygen saturation of hemoglobin (SpO_2) nadir, %*	84.9 ± 4	93.8 ± 2.5	94.6 ± 2.4
Mean SpO_2, %†	95.5 ± 2.2	96.8 ± 0.8	97.4 ± 0.7
Percent sleep time with SpO_2 <90%‡	1.4 (0.2-3.4)	0.4 (0-2)	0.1 (0-1)
Total sleep time, min	405.3 ± 91.7	398.1 ± 90.6	357.8 ± 64.2
Sleep efficiency, %	82 ± 13.3	81.8 ± 11.2	77.7 ± 11.2
Stage N1, % of total sleep time	13.8 ± 14	12.7 ± 8.1	18.2 ± 10.6
Stage N2, % of total sleep time†	54 ± 12.4	45.3 ± 12.5	42.2 ± 9.4
Stage N3, % of total sleep time†	17.2 ± 8.2	22.4 ± 6	23.9 ± 7.9
Stage REM, % of total sleep time	16.9 ± 6.1	20.4 ± 8	15.8 ± 5.1
Poincaré plot width (ms)§	318.7 ± 139.3	399.5 ± 180.6	484.2 ± 104.4
Ln-transformed urine norepinephrine§	4.5 ± 0.6	4.2 ± 0.6	3.8 ± 0.4

Abbreviations:
BMI = Body mass index
OSA = Obstructive sleep apnea
REM = Rapid eye movement
Resp. = Respiratory
Continuous variables are expressed as mean ± standard deviation or median (interquartile range).
* $P < 0.01$ for children with OSA and moderate-to-severe hypoxemia versus mild hypoxemia or control subjects.
† $P < 0.05$ for children with OSA and moderate-to-severe hypoxemia versus mild hypoxemia or control subjects.
‡ $P = 0.01$ for children with OSA and moderate-to-severe hypoxemia versus control subjects (significant after Bonferroni correction).
§ $P < 0.01$ for children with OSA and moderate-to-severe hypoxemia versus control subjects.

Results

A total of 55 children were recruited, 44 of whom were children with OSA and 11 were control subjects. Twenty participants with OSA had moderate-to-severe hypoxemia and the remaining had mild hypoxemia. The three study groups were similar regarding subjects' characteristics (Table 1). Children with OSA and moderate-to-severe hypoxemia had substantially worse polysomnography indices than those with mild hypoxemia or control participants ($P < 0.01$; Table 1). The three study groups were significantly different regarding percent sleep time with SpO_2 <90% ($P = 0.04$). AHI >10 episodes/hr (severe OSA) was noted in 14 children, and all of them had episodes of oxyhemoglobin desaturation with median desaturation index of 13.8 episodes/hr (range 8-71.6 episodes/hr).

Mean urine norepinephrine-to-creatinine ratios were 106.9 ± 84.7, 80.9 ± 48.3, and 47.9 ± 19.9 ng/mg of urine creatinine in children with OSA and moderate-to-severe hypoxemia, subjects with OSA and mild hypoxemia, or control participants, respectively. The three groups differed significantly in terms of the Poincaré plot width ($P = 0.02$) and ln-transformed urine norepinephrine-to-creatinine ratio ($P = 0.01$). Children with OSA and moderate-to-severe hypoxemia had significantly narrower plots and higher norepinephrine concentrations than control subjects (Table 1 and Figs 2 and 3).

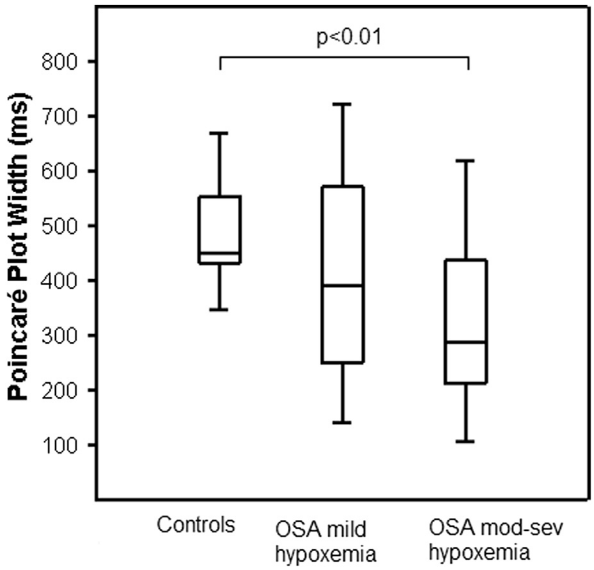

FIGURE 2.
Box plots of the Poincaré plot width in the three study groups. Children with obstructive sleep apnea (OSA) and moderate-to-severe nocturnal hypoxemia (SpO_2 nadir <90%) did not differ regarding the Poincaré plot width from those with OSA and mild nocturnal hypoxemia (SpO_2 nadir $\geq$90%) but had significantly narrower width than control subjects ($P < 0.01$). Horizontal lines within the box plots represent median values. mod-sev, moderate to severe; SpO_2, oxygen saturation of hemoglobin.

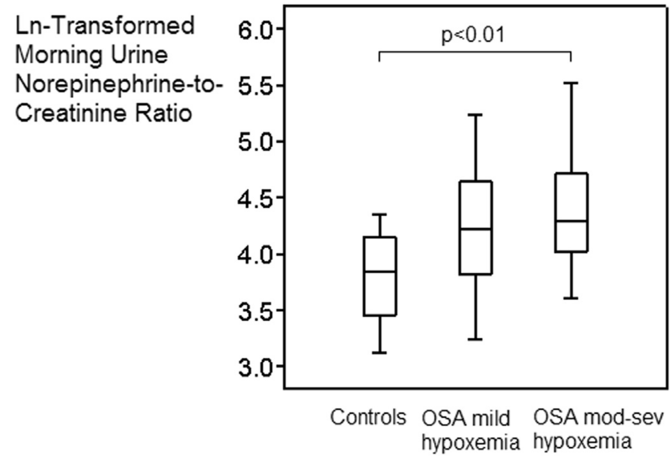

FIGURE 3.
Box plots of ln-transformed morning urine norepinephrine-to-creatinine ratio in the three study groups. Children with obstructive sleep apnea (OSA) and moderate-to-severe nocturnal hypoxemia (SpO_2 nadir <90%) did not differ regarding urine norepinephrine level from those with OSA and mild nocturnal hypoxemia (SpO_2 nadir $\geq$90%) but had significantly higher morning urine norepinephrine level than control subjects ($P < 0.01$). Horizontal lines within the box plots represent median values. mod-sev, moderate to severe; SpO_2, oxygen saturation of hemoglobin.

Width of the Poincaré plot was positively associated ($r = 0.37$, $P < 0.01$) and ln-transformed urine norepinephrine concentration was negatively associated with SpO_2 nadir ($r = -0.34$, $P = 0.01$) even after adjustment for age (Table 2). Poincaré plot width was negatively related to respiratory arousal index ($r = -0.29$, $P = 0.03$) and AHI ($r = -0.36$, $P < 0.01$; Table 2). No significant associations were demonstrated between ln-transformed urine norepinephrine concentration and respiratory arousal index ($r = 0.05$, $P > 0.05$), whereas the association with AHI approached statistical significance ($r = 0.25$, $P = 0.07$).

An inverse function described the relationship between heart rate variability and urine norepinephrine ($r^2 = 0.10$, $P = 0.02$; Fig 4):

$$\text{ln} - \text{transformed urine norepinephrine} - \text{to} - \text{creatinine ratio} = 3.86 + [113.492/(\text{width of the Poincaré plot})]$$

Discussion

In the present investigation, an index of nocturnal parasympathetic activity, i.e., the width of the Poincaré plot derived from the electrocardiographic channel of polysomnography during the whole night of sleep, and a measure of nocturnal sympathetic output, i.e., morning urine norepinephrine levels, were applied to demonstrate autonomic imbalance in children with OSA. Both measures are readily available in most hospital-based polysomnography systems and hospital biochemistry laboratories, and they can easily be used by clinicians in contrast to more complex methods such as spectral analysis of the heart rate variability.

Heart rate is the inverse of the R-R interval length on the electrocardiogram (frequency = 1/period), and heart rate variability represents the continuous oscillation of the R-R interval length around its mean value (i.e., the variability of the intervals between successive heart beats).[9] Sympathetic and parasympathetic outputs modulate heart rate variability.[14] Therefore, indices of R-R interval variability calculated by complex mathematical analysis (spectral analysis) of the electrocardiogram can be used as indirect measures of the sympathetic and parasympathetic activity.[14,15]

Analysis of the Poincaré plot which is a scattergram of the current R-R interval ($R\text{-}R_{n+1}$) against the preceding R-R

TABLE 2.
Multiple Linear Regression Analysis Models for the Evaluation of the Independent Effect of Polysomnography Indices on the Width of the Poincaré Plot and on Ln-Transformed Morning Urine Norepinephrine-to-Creatinine Ratio in Children With Obstructive Sleep Apnea and Control Subjects

Independent Variables	β (Standardized Coefficient)	*P* Value
Model 1. Dependent variable: width of the Poincaré plot (adjusted $r^2 = 0.14$; $P = 0.02$)		
Age	0.07	NS
SpO_2 nadir	0.38	<0.01
Model 2. Dependent variable: width of the Poincaré plot (adjusted $r^2 = 0.06$; $P = 0.08$)		
Age	0.08	NS
Respiratory arousal index	−0.30	0.03
Model 3. Dependent variable: width of the Poincaré plot (adjusted $r^2 = 0.10$; $P = 0.03$)		
Age	0.08	NS
Apnea-hypopnea index	−0.37	<0.01
Model 4. Dependent variable: ln-transformed morning urine norepinephrine-to-creatinine ratio (adjusted $r^2 = 0.39$; $P < 0.01$)		
Age	−0.53	<0.01
SpO_2 nadir	−0.39	<0.01

Abbreviation:
NS = Nonsignificant

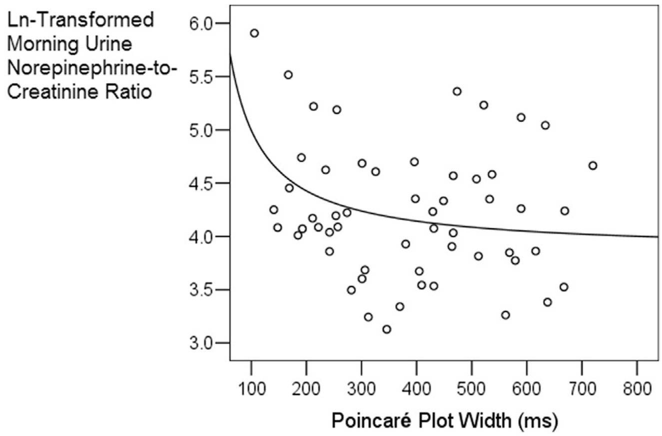

FIGURE 4.
Width of the Poincaré plot was inversely related to ln-transformed morning urine norepinephrine-to-creatinine ratio ($r^2 = 0.10$, $P = 0.02$).

interval (R-R_n) is an alternative and simpler method. The width of the plot corresponds to the variability of the R-R interval around the line of identity (diagonal line at 45 degrees to both axes) and correlates with the parasympathetic nervous system activity (Fig 1).[8] On termination of an obstructive apnea or hypopnea, sympathetic activity increases and parasympathetic output decreases. The R-R interval on the electrocardiogram becomes shorter compared with baseline (faster heart rate), its variability decreases, and blood pressure rises transiently.[16,17] In other words, the time period between the heart beats (n-1) and n becomes approximately equal to the time period between the heart beats n and (n+1) because of decreased parasympathetic output. As a result, the point corresponding to (R-R_{n+1}) plotted against (R-R_n) will be close to the line of identity, and if such apneic events occur frequently the Poincaré plot will be narrow in shape (Fig 1).

In addition, catecholamine concentrations can be used as surrogate markers of sympathetic tone during the collection period of the urine sample.[18] Enhanced urine excretion of norepinephrine in children with OSA has been well described.[6,19,20] In the present report, a weak but significant inverse association between a surrogate measure of parasympathetic tone (Poincaré plot width) and an index of nocturnal sympathetic activity (morning urine norepinephrine) has been demonstrated, which supports further the concept of autonomic imbalance in children with OSA (sympathetic overflow not balanced by the parasympathetic system).[2]

Previous studies have demonstrated that autonomic imbalance persists even between episodes of upper airway obstruction and also during wakefulness.[9,17] Several pathophysiologic mechanisms have been proposed to interpret the effects of OSA on the autonomic nervous system. Sympathetic nerve activity, heart rate, and levels of blood pressure decline from wakefulness to non−rapid eye movement sleep and increase again during rapid eye movement sleep.[21] Arousals from sleep—such as those accompanying obstructive events—are associated with bursts of sympathetic nerve activity.[12,21] Sympathetic tone can be also augmented by hypoxemia and hypercapnia related to apneas and hypopneas via stimulation of peripheral and central chemoreceptors.[13] Hence, it has been postulated that intermittent gas exchange abnormalities and arousals associated with intermittent upper airway obstruction during sleep enhance the sympathetic activity, which remains increased even during normoxic wakefulness.[22-25] Of note, low SpO_2 nadir in the present study correlated with high urine norepinephrine concentration (increased sympathetic activity) and narrow Poincaré plot (reduced parasympathetic tone). In addition, width of the plot was negatively related to respiratory arousal index.

Reduced heart rate variability along with repetitive rises in blood pressure and resetting of the baroreceptors might predispose to hypertension and cardiovascular morbidity in adult life.[26,27] Although treatment of OSA in children by adenotonsillectomy improves somatic growth rate, nocturnal enuresis, behavior, quality of life, and polysomnographic findings, its effects on OSA-related cardiovascular morbidity are less clear.[28,29] A large proportion of children with habitual snoring and adenotonsillar hypertrophy who are referred for polysomnography have only mild sleep-disordered breathing (AHI 1-5 episodes/hr) and absence of delayed growth rate, enuresis, or cognitive and behavioral abnormalities. In such cases, indications for adenotonsillectomy are obscure. Autonomic nervous system abnormalities are of potential clinical importance because they resolve as soon as 4 months after adenotonsillectomy and could potentially be used as outcome measures in future clinical trials evaluating treatment interventions for mild sleep-disordered breathing.[27,30,31]

Conclusions

Poincaré plot width is reduced and morning urine norepinephrine concentration is increased in children with OSA and moderate-to-severe nocturnal hypoxemia, and they are inversely related to each other, most likely reflecting autonomic nervous system imbalance. Both measurements are readily available in pediatric sleep centers and simpler than other methods used for demonstrating autonomic imbalance.

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